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14. ABSTRACT There are reports in the literature showing one of the essential amino acids (AAs), leucine, playing a role in enhancing cognition and memory in animal and man, partially via the mammalian target of rapamycin (mTOR) signaling pathway regulating neuronal protein synthesis and plasticity. This pathway is a potential target for modulation with leucine (or other therapeutic agents), to maintain/enhance normal functioning under stress conditions. Such an effect has potential for optimizing warfighter cognitive performance under high demand conditions. The kinetic behavior of leucine is, however, not well characterized. Most published studies involve uptake of leucine-enhanced drinking water or diet, and concentrations of leucine in tissues are typically measured at only one time point at the study's end (Tews and Harper 1991). To fill these data gaps and to develop a physiologically-based pharmacokinetic (PBPK) leucine model, an <i>iv</i> leucine study was performed in Long-Evans rats, collecting both time-course and dose-response blood and tissue data. Such a PBPK model, once validated, will allow extrapolation of leucine dosing and intake across dosing regimens (drinking water, diet) and across species to predict potential brain concentration changes in man. Brain concentrations in both animals and man can then be linked with potential impacts on the mTOR signaling pathway, and on observed cognitive changes. Preliminary results revealed that leucine is eliminated from the blood very quickly after <i>iv</i> dosing (5mg/kg), and leucine levels in the brain were higher than blood, indicating active transport of leucine across the blood-brain-barrier (BBB). At 5 min post dosing, only 7% of injected leucine was detected still in blood, and at 6 hr post doing it was down to base line levels. Similarly, of the 17 other AAs measured in brain, about 2/3 were increased, even at very early times (<2 min) after <i>iv</i> leucine injection. Most of those that either remained unchanged or were increased in brain are known to be transported across the BBB by the L1 large neutral AA transporter (Smith, 2000; Wade, 1981). Thus, since this system is normally close to saturation, and since leucine has a high affinity for this transporter, one may speculate that the spike in leucine levels due to the <i>iv</i> injection tends to cause an increased brain influx of ALL AA's, but that competition for the L1 transporter mitigates this effect somewhat for many of the AAs that primarily make use of this system to enter the brain.					
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Kinetic Behavior of Leucine and Other Amino Acids Modulating Cognitive Performance via mTOR Pathway

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ABSTRACT

There are reports in the literature showing one of the essential amino acids (AAs), leucine, playing a role in enhancing cognition and memory in animal and man, partially via the mammalian target of rapamycin (mTOR) signaling pathway regulating neuronal protein synthesis and plasticity (Hoeffler and Klann, 2009; Sarbassoy et al 2005; Tokunaga et al 2004). This pathway is a potential target for modulation with leucine (or other therapeutic agents), to maintain (or even enhance) normal functioning under stress conditions. Such an effect has potential for optimizing warfighter cognitive performance under high demand conditions. The kinetic behavior of leucine is, however, not well characterized. Most published studies involve uptake of leucine-enhanced drinking water or diet, and concentrations of leucine in tissues are typically measured at only one time point at the study's end (Tews and Harper 1991). To fill these data gaps and to develop a physiologically-based pharmacokinetic (PBPK) leucine model, an *iv* leucine study was performed in Long-Evans rats, collecting both time-course and dose-response blood and tissue data. Such a PBPK model, once validated, will allow extrapolation of leucine dosing and intake across dosing regimens (drinking water, diet) and across species to predict potential brain concentration changes in man. Brain concentrations in both animals and man can then be linked with potential impacts on the mTOR signaling pathway, and on observed cognitive changes. Preliminary results revealed that leucine is eliminated from the blood very quickly after *iv* dosing (5mg/kg), and leucine levels in the brain were higher than blood, indicating active transport of leucine across the blood-brain-barrier (BBB). At 5 min post dosing, only 7% of injected leucine was detected still in blood, and at 6 hr post doing it was down to base line levels. Similarly, of the 17 other AAs measured in brain, about 2/3 were increased, even at very early times (<2 min) after *iv* leucine injection. Most of those that either remained unchanged or were increased in brain are known to be transported across the BBB by the L1 large neutral AA transporter (Smith, 2000; Wade, 1981). Thus, since this system is normally close to saturation, and since leucine has a high affinity for this transporter, one may speculate that the spike in leucine levels due to the *iv* injection tends to cause an increased

brain influx of ALL AA's, but that competition for the L1 transporter mitigates this effect somewhat for many of the AAs that primarily make use of this system to enter the brain.

INTRODUCTION

- Leucine, an essential amino acid, has been linked with cognitive enhancement in both experimental animals and humans
- Leucine is involved in the regulation of the mTOR (mammalian target of rapamycin) pathway
- The mTOR pathway regulates protein synthesis in cells, including neurons
- Neuronal protein synthesis is associated with synaptic plasticity and memory consolidation
- There is a need to link *in vivo* leucine exposures, via drinking water, diet, and other routes, with brain leucine levels, in order to develop dosing regimens with the potential for cognitive enhancement in humans (dose-response).
- Development of mechanism-based mathematical models, including a physiologically-based pharmacokinetic (PBPK) model for leucine will help provide such a (quantitative) link
- In order to develop such a model for leucine, we performed an iv study in rats, in which leucine was measured in blood, brain and other tissues at various times after injection.
- Since leucine is part of a dynamic system involving other amino acids, which interact with leucine in various ways (such as competition for transport across the blood-brain barrier – see Table 1), other amino acid blood and brain levels were also measured.

Amino Acid	BBB Transporter		Type		Structure
Leucine	L1	Essential	Neutral	Non-polar (hydrophobic)	Branched chain
Aspartic Acid	Acidic X-		Acidic	Polar	
Glutamine	Acidic X-		Neutral	Polar	
Serine	ACS		Neutral	Polar	
Histidine	L1	Essential	Basic	Polar	Aromatic
Glycine	L1		Neutral	Non-polar	
Cysteine	ACS		Neutral	Slightly polar	
Arginine	Basic Y+		Basic	Polar	
Alanine	ACS		Neutral	Non-polar	Aromatic
Tyrosine	L1		Neutral	Polar	Aromatic
Threonine	L1	Essential	Neutral	Polar	
Valine	L1	Essential	Neutral	Polar	Branched chain
Methionine	L1	Essential	Neutral	Non-polar	
Phenylalanine	L1	Essential	Neutral	Non-polar	Aromatic
Isoleucine	L1	Essential	Neutral	Non-polar	Branched chain
Lysine	Basic Y+	Essential	Basic	Polar	
Proline	L1?		Neutral	Non-polar	Aromatic
Asparagine			Neutral	Polar	
Glutamic Acid			Acidic	Polar	
Tryptophan	L1	Essential	Neutral	Slightly polar	Aromatic

Table 1: Properties of amino acids and associated BBB transport systems (Those in gray cells were not measured)

EXPERIMENTAL (preliminary studies)

- Species: Male Long-Evans rats, n=3
- Dosing levels: 5 and 12.9 mg/kg leucine in physiological saline
- Dosing volume: 1.5 mL/kg
- Dosing route: Indwelling jugular vein catheter
- Time points: 0, 2, 4, 5, 15, and 30 min, 1, 3, and 6h post dosing
- Tissues collected: blood, brain, kidney, liver, muscle, brown and white fat
- Method to isolate AAs: Homogenization, TCA treatment to precipitate protein, centrifugation, and injection of supernatant
- Amino acids analysis condition:

Instrument: Agilent 1100 series liquid chromatography system coupled with fluorescent detection (EX = 250, EM = 410 nm)

Column: Zorbax Eclipse AAA columns, 4.6*150 mm, pore size – 5 micron

Mobile Phase: A: 40mM NaH₂PO₄ pH 7.8, B: (Acetonitrile:methanol: water = 45:45:10 v/v/v), Starting 100% A and gradually increase up to 100% B in 55 min)

Amino acid STD: Factory provided standards of 17 amino acids were used (see Table1)

RESULTS

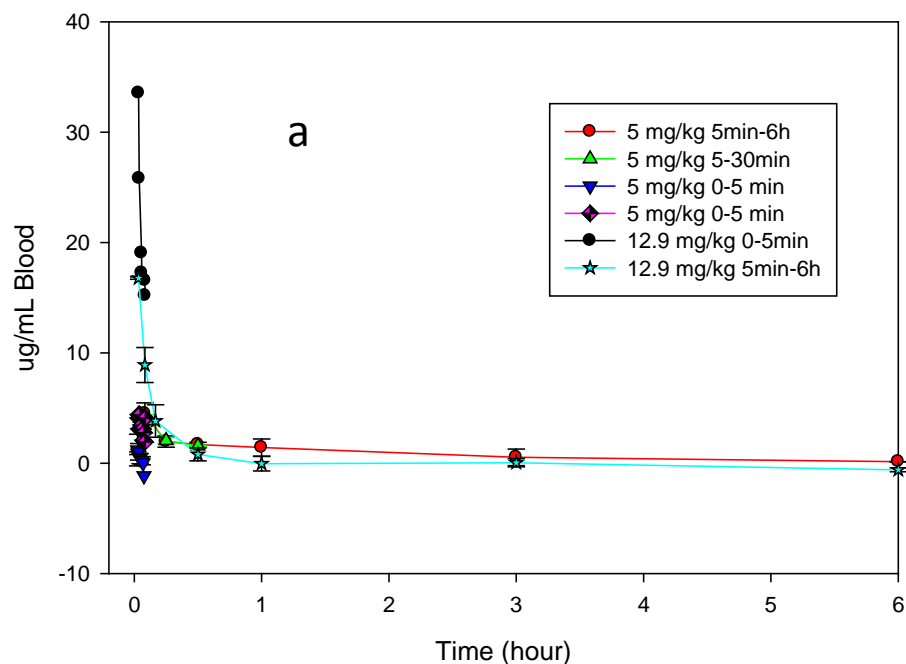


Figure 1. Leucine concentration in blood of rats dosed with leucine (5 and 12.9mg/kg). Rats (n=3) were injected with 5 & 12.9 mg/kg leucine via indwelling catheter, and tissue samples were collected 0-6h post dosing (**Fig 1a**). Leucine was eliminated very fast from the circulating blood. Control values were subtracted. **Fig 1b** shows early times.

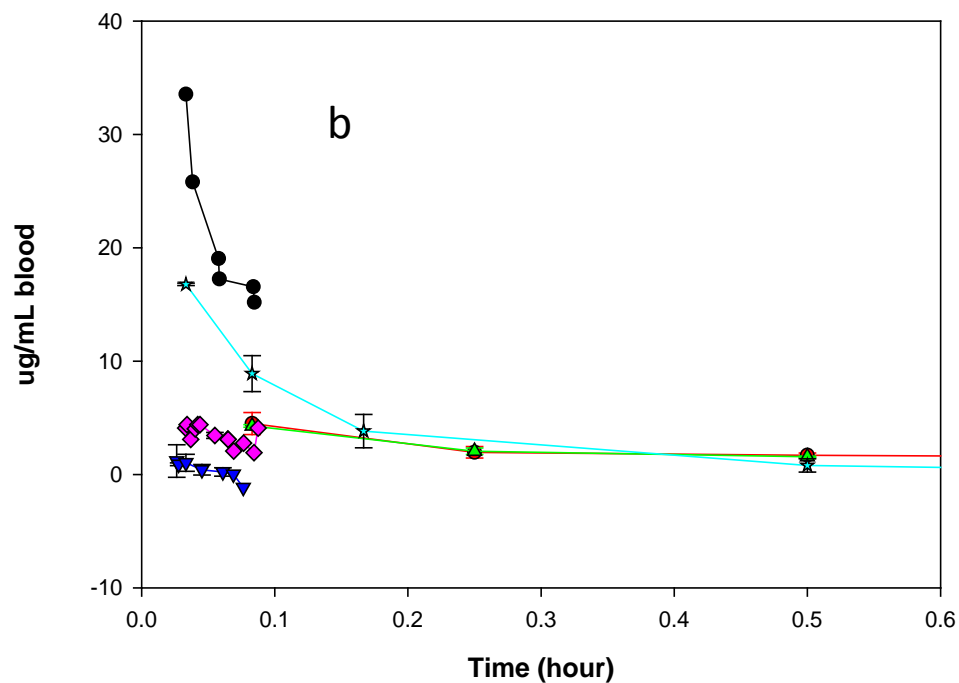


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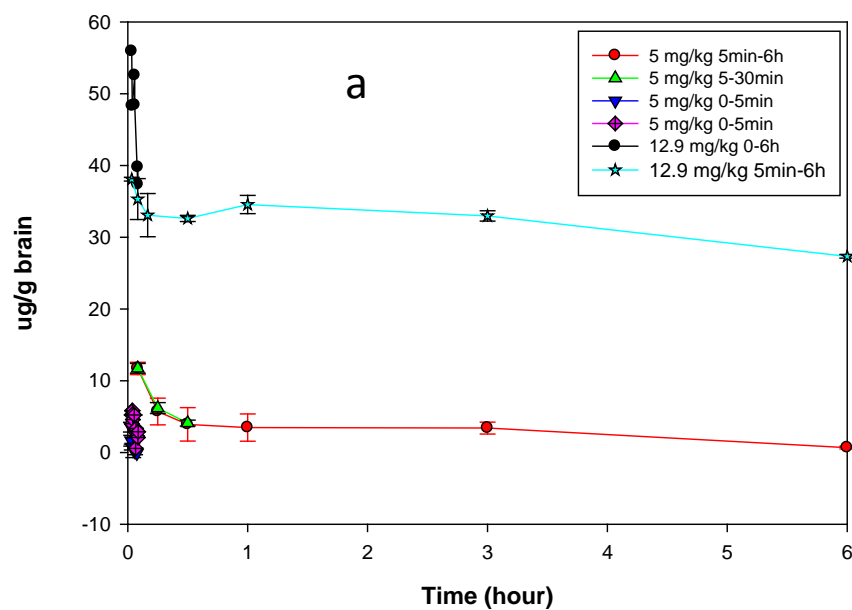


Figure 2. Leucine concentration of leucine in rat's brain. Rats (n=3) were injected with 5 & 12.9 mg/kg leucine via indwelling catheter, and brain samples were collected 0-6h post dosing (**Fig 2a**). Control values were subtracted. **Fig 2b** shows early times.

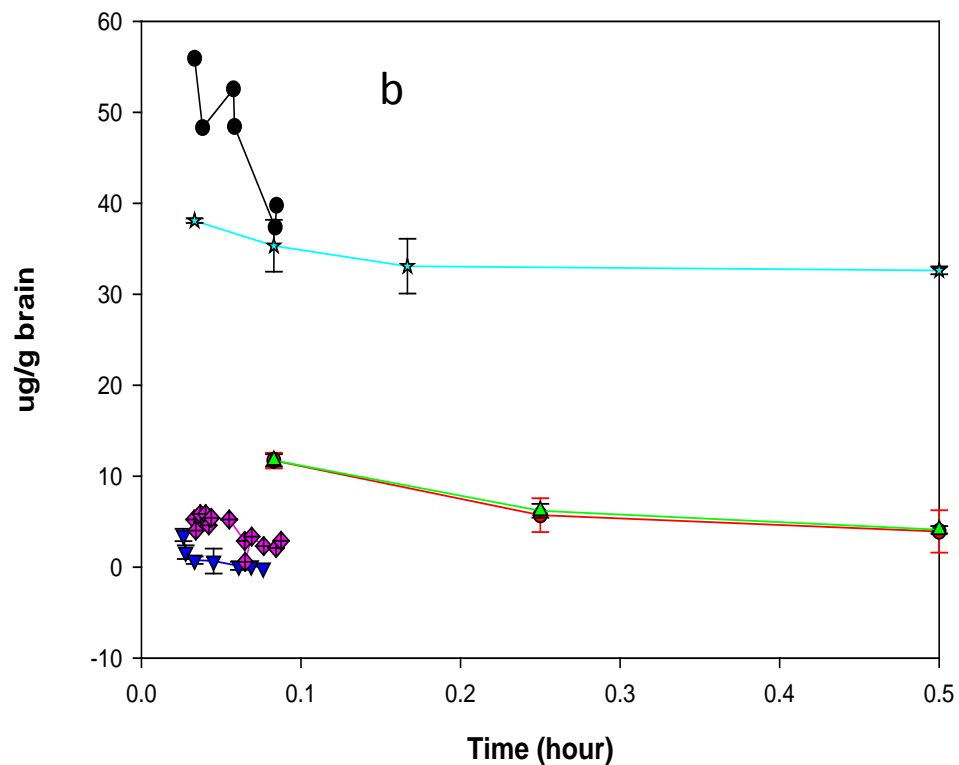


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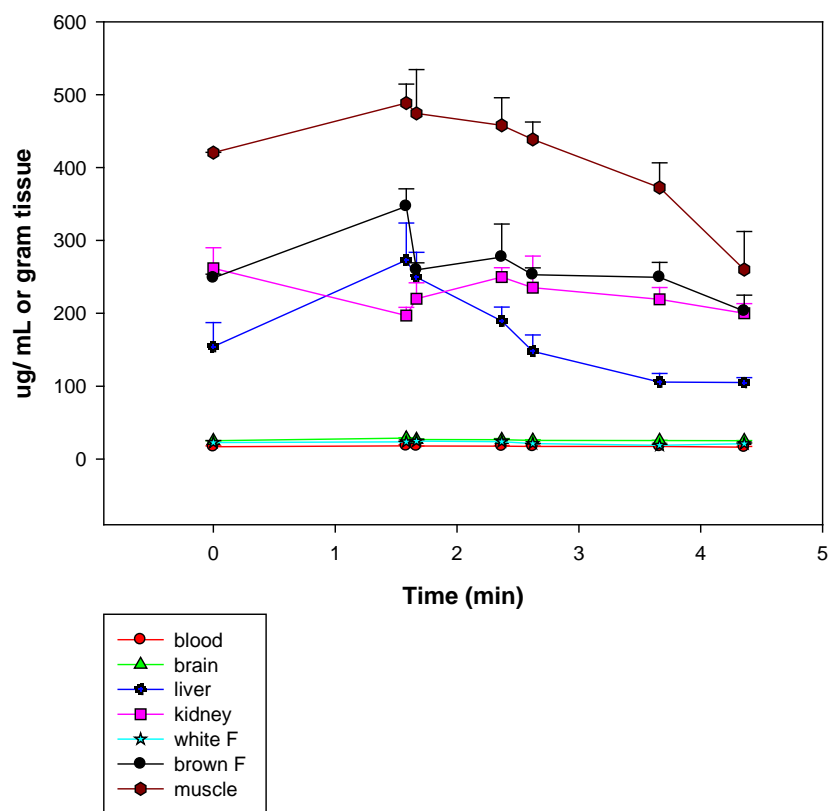


Figure 3. Leucine concentrations in tissues of rats dosed with 5mg/kg leucine. Rats (n=3) were injected with leucine and tissues were collected 0-4.5h post dosing. Control values were subtracted.

plasma 5 mg/kg			brain 5 mg/kg				
methionine ↑	serine ↓	histidine ↓	arginine ↑	proline ↑	isoL ↑	aspartic A ↑	lysine ↑
7	10	6	91	84	81	56	52
			histidine ↑	methionine ↑	valine ↑	glutamic A ↑	thyrrosine ↑
			44	41	36	34	27

plasma 12.9 mg/kg			brain 12.9 mg/kg			
isoL ↑	glycine ↑	threonine ↓	lysine ↑	serine ↑	proline ↑	threonine ↓
23	18	27	60	59	44	24

Table 2. Percentage increase or decrease of other amino acids: following iv leucine injection. Rats were dosed with two doses (5 & 12.9 mg/kg) of leucine, and blood and brain were harvested at different time points (0-6h). Effects of leucine on the other amino acids were compared. Percentage of increase or decrease were reported as the average values of 6 time points.

CONCLUSION

- Clearance of leucine in blood is very fast after iv dosing (both 5 and 12.9 mg/kg).
- At 5 min post dosing, only 6.7% for 5mg/kg and 7.4% for 12.9 mg/kg of the original doses were found in blood. By 10 min post dosing, both reached to the same levels of leucine in blood.
- Leucine levels in brain were higher than blood indicating active transport across the blood brain barrier.
- Leucine concentration in brain of rats dosed with 12.9 mg/kg maintained high concentration (~29 ug/g brain) at the end of the experiment, while low dose (5 mg/kg) reached ~3 ug/g.
- Time course of tissue concentration revealed that muscle had the highest concentration of leucine followed by brown fat, kidney and liver. Blood, brain and white fat had low levels of leucine compared with other tissues.
- Effects of leucine on other amino acids were analyzed. Those measured were aspartic acid, glutamic acid, serine, histidine, glycine, alanine, cysteine, phenylalanine, isoleucine, lysine, threonine, arginine, tyrosine, valine, methionine and proline.
- Table 2 shows that low dose (5mg/kg) of leucine effects on plasma was not strong compared with brain. Percentages of ten amino acids were increased while 2 amino acids in plasma were decreased.
- High dose (12.9 mg/kg) of leucine in plasma produced less effects compared with brain. Threonine levels were decreased in both plasma and brain.
- Both lysine and proline levels in brain of rats dosed with 5 & 12.9 mg/kg leucine produced strong effects for these amino acids

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